

Mechanisms of Resistance in the Oral Microflora of Periodontitis Patients.  
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Antibiotic therapy has been used as an adjunct to both scaling, root planing and surgery, in the treatment of periodontal disease. Preliminary findings suggest that refractory periodontitis, defined as the failure to arrest the progression of periodontitis using conventional therapeutic strategies, could be related to antibiotic resistant periodontal pathogens. We have cultured two patients who had antibiotic therapy for their disease but had been off antibiotics a year prior to obtain their cultures. In the first patient, tetracycline resistant ( $Tc^r$ ) bacteria represented 10% of the total cultureable bacteria isolated. Twenty three  $Tc^r$  strains were identified from 27 isolates. These included gram-positive, gram-negative, aerobic and anaerobic species and were represented by *Haemophilus*, *Neisseria*, *Fusobacterium nucleatum*, *Prevotella intermedia*, as well as, a variety of streptococci. All but three isolates were shown to hybridize with one or more of the characterized Tet genes and 13 of these isolates hybridized with one or more of the characterized rRNA methylase genes which confer erythromycin resistance ( $Em^r$ ). Two *H. aphrophilus* and one *Neisseria sicca/perflava* were resistant to penicillins. The *Neisseria* and one *Haemophilus* isolate had nonezymatic resistance, while the other *Haemophilus* carried a  $\beta$ -lactamase enzyme. We also selected two *Bacteroides forsythus*, and four *Campylobacter (Wolinella) recta* isolates from the plain agar plates and demonstrated that each of these isolates hybridized with one or more of the rRNA methylase gene probes examined. Many of the 33 isolates examined could transfer the Tet and/or Erm determinants to recipients in the laboratory, by conjugation. In the second patient, 51 isolates were screened with tetracycline and erythromycin DNA probes and 22 hybridized with one or more of the probes examined. Our data indicates that a significant number of the oral flora, found in pockets of the two refractory periodontitis patients, are resistant or multiply resistant to antibiotics used in each patients treatment. The genes they carry are, in most cases, those that have been previously characterized in  $Tc^r$  and  $Em^r$  gram-positive and gram-negative species. The presence, after a year without antibiotic therapy, of both antibiotic resistant normal flora and pathogens suggests the relative stability of these resistant species in the patients investigated. Whether antibiotic resistant pathogens play a role in refractory periodontitis, needs further study, but our data is consistent with that hypothesis. The presence and donorability of multiple antibiotic resistance genes suggest that these bacteria could act as an antibiotic resistance gene reservoir in these patients. This could in turn impact further effort in preventing their periodontal disease from progressing.